G. C. Schmidt and T. E. Eling

AFOSR 67-2030

Final Scientific Report for [redacted] AFOSR-67-66

Title: Synthesis of Atropine Labeled with C^{14} in the Tropine Ring and Effect of Cold Acclimation on Atropine Metabolism.

Bibliography:

1. Schmidt, G.S., Eling, T.E. and Drach, J.C.: Synthesis of Tropine-Labeled Atropine. I. Micromethods for the Synthesis of Tropine and for its Esterification with Tropic Acid. J. Pharmaceut. Sci. 56: 215 - 221, 1967.

2. Kalser, S.C. and Kunig, R.: Effect of Varying Periods of Cold Exposure on the Action and Metabolism of Hexobarbital. Submitted to J. Pharmacol. exp. Ther. in May, 1967.

3. Kalser, S.C., Evans, E., Forbes, E., Kelly, M., Kelvington, E., Kunig, R. and Randolph, M.: Decreased Atropine Toxicity in Rats Chronically Exposed to Cold. To be submitted to Toxicology and Applied Pharmacology, August, 1967.

4. Schmidt, G.S. and Eling, T.S.: Progress report on Synthesis of Ring-labeled Atropines. Several papers to be published when syntheses completed.

5. Kalser, S.C. and McLain, P.L.: Tropine-labeled Atropine Metabolism in Man: N-$C^{14}H_3$-Atropine. Progress report, paper to be published when study completed.

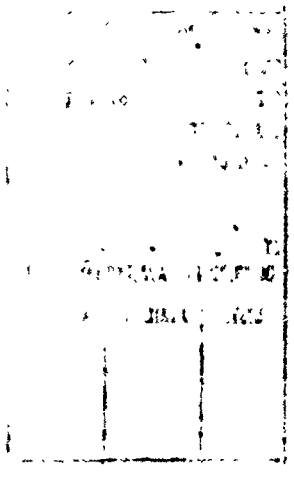
Summary of Results of Research accomplished under Grant AFOSR 87:

Synthesis of a series of C^{14}-tropine-labeled atropines, which have previously been unavailable, has been accomplished. A few of these compounds are still being synthesized, but when the series is completed, it will represent the most thorough analysis of the tropine ring to be undertaken. Every carbon in the ring will have been tagged separately or in various combinations. In addition, the N-$C^{14}H_3$-atropine has also been prepared and made available.

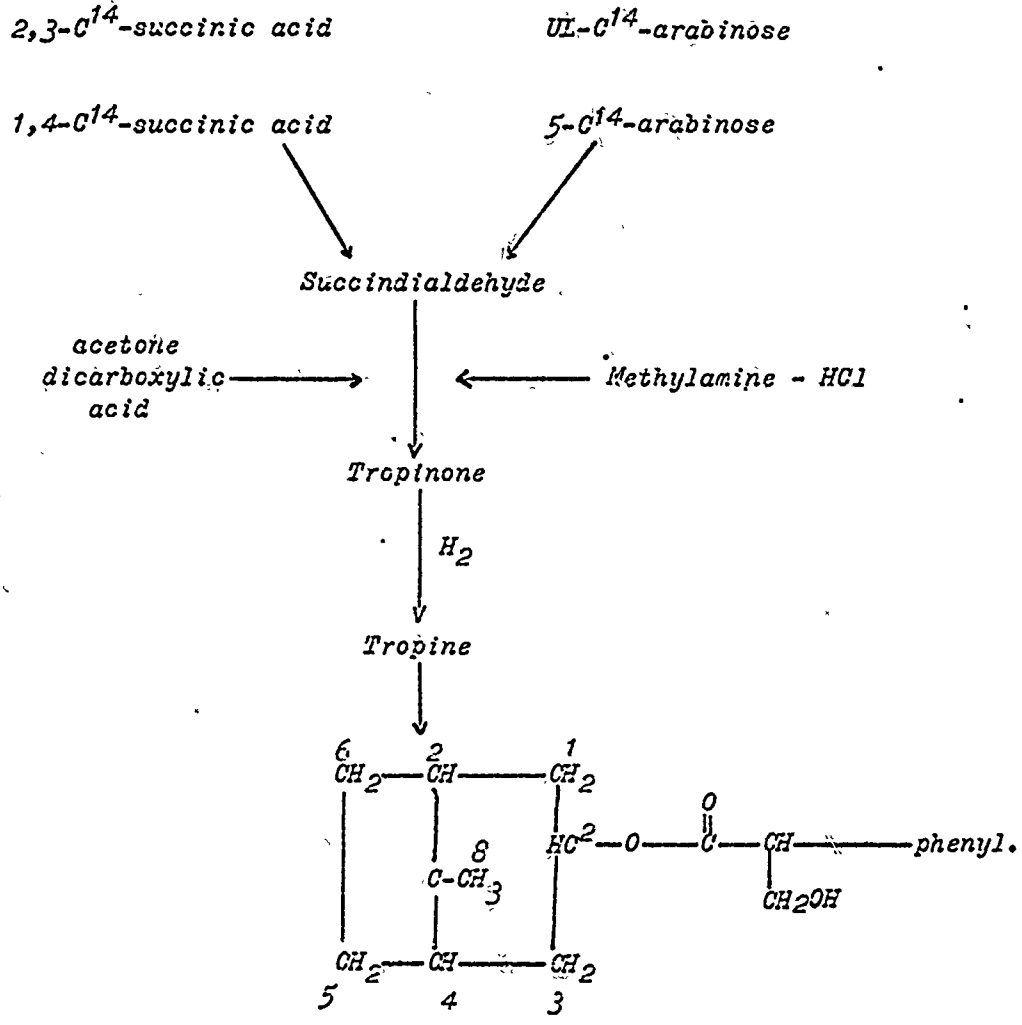
We have shown that environmental conditioning to cold can almost double the LD_{50} for atropine compared with the LD_{50} of control rats; however, an acute exposure to cold decreases the LD_{50}. These changes in toxicities parallel the rate of absorption of atropine from the peritoneal cavity; the rate of absorption is slowest in the cold-acclimated and fastest in the rat exposed to cold acutely. Excretion and metabolism of atropine is not increased in the cold-acclimated rat. This suggests that the rate of absorption of atropine probably modulates the toxicity pattern of the drug.

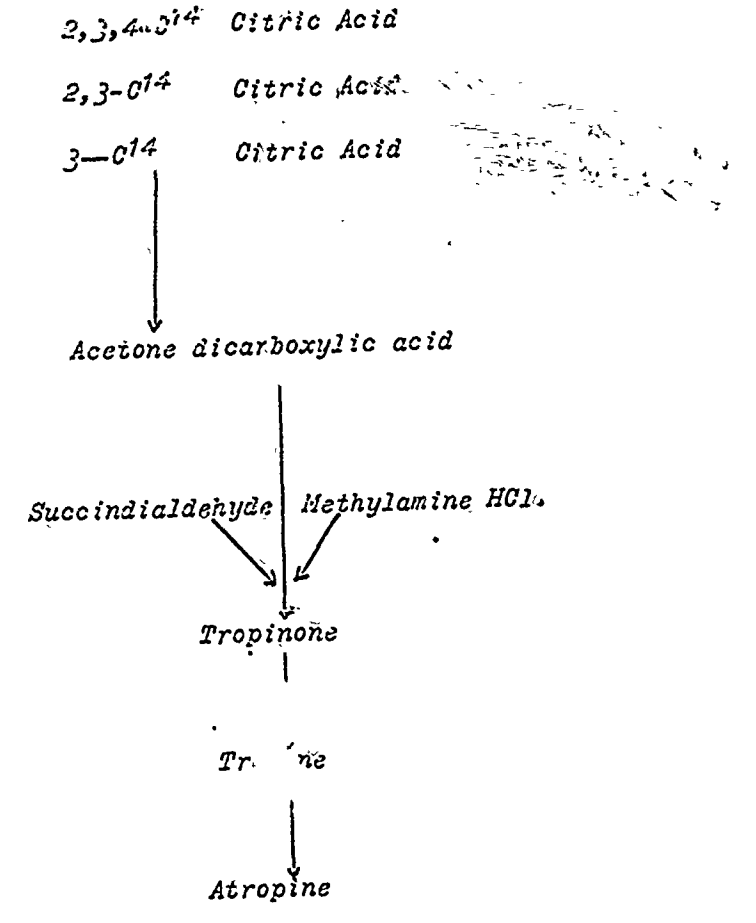
Using an N-$C^{14}H_3$-atropine, a study in normal man showed that 91% of the i.m. dose was excreted; 88% in the urine in 48 hours and 3 % in the expired air in 3 hours. This suggests that demethylation of atropine occurs as one of the metabolic pathways for

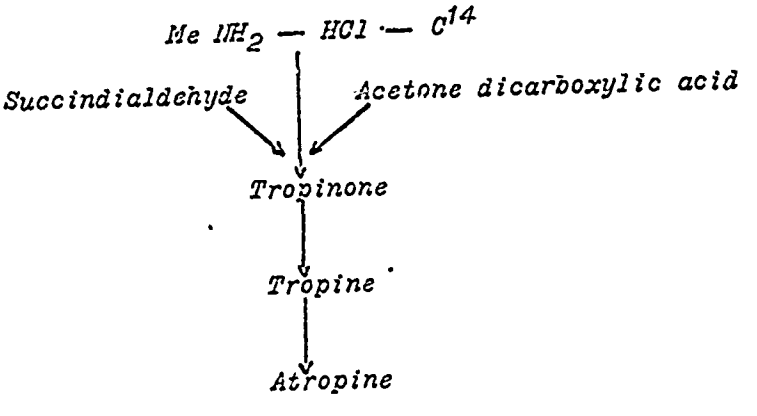
degradation in man. Most of the urinary products appear to be the unchanged drug and tropine, in about equal amounts. However, this phase of the study is still in its preliminary stage. The drug disappears exceedingly rapidly from the blood. The urinary excretion pattern suggests at least 3 rates of clearance. Since one of these rates may relate to an enterohepatic phase of recirculation of the drug, biliary excretion will be studied in man. At least a few of the ring-labeled atropines will also be studied in man.



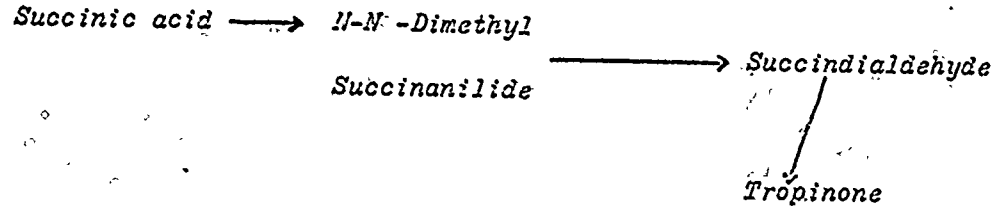
The objective of this investigation was to synthesize atropine labeled with C^{14} in the tropine moiety. The proposed method for selective labeling of the tropine ring of atropine is outlined in the schemes below.




2,3,4-C14 Citric Acid
2,3-C14 Citric Acid
3-C14 Citric Acid
Acetone dicarboxylic acid
Succindialdehyde
Methylamine HCl
Tropinone
Tropine
Atropine


MeNH2 — HCl — C14
Succindialdehyde
Acetone dicarboxylic acid
Tropinone
Tropine
Atropine

During the first year, the investigation was directed to scaling down from 5 gm. to 300-400 mg level the reaction sequence (see below) from succinic acid to succindialdehyde.



From 354 mg of succinic aicd, tropinone was obtained in yields ranging from 65-85 mg. The percentage yield of tropinone based on succinic aicd averaged approximately 20%.

Detailed studies for the formation of tropinone from precursor, conversion of tropinone to tropine, and formation of atropine via the esterification of tropine with tropic acid was also conducted. This work, published in the Journal of Pharmaceutical Science, 1967, established the reproducibility of these steps which is required for C^{14} labeling of the tropine moiety of atropine.

The primary objective of the second year was the labeling of atropine. During this year, problems arose in the reduction of tropinone to atropine which delay labeling. Our original method for reduction was discarted, and a new procedure for reduction developed.

Since the cheapest C^{14} starting material was methylamine -C^{14} HCl., this labeled intermediate was first used in order to check the entire procedure for possible new problems. After five unsuccessful labeling attempts, N-Me^{14} atropine was pooled, the C^{14} tropinone isolated and converted to

N-Me^{14} tropine. This procedure resulted in the recovery of 35 mg of N-Me^{14} tropine which could be used in limited metabolic studies and for standards on chromatograms.

Two attempts at labeling atropine are in progress at grant termination. As of this date we have 50 mg of 3-C^{14} tropine and are preparing for esterification with tropic acid to form 3-C^{14} atropine. Also 2,3,4-C^{14} tropinone has been synthesized but the yield has not been determined. This labeled intermediate is in the process of purification before subsequent conversion to 2,3,4-C^{14} atropine.

We feel that the synthesis has reached the point where it is just a matter of "grinding out" the C^{14} labeled compounds. The method from citric acid appears to be working and the method from succinic acid, using cold starting materials, has worked well. The following labeled atropines are expected to be available in a few months:

Labeled Intermediate	Position of Label in Atropine
3-C^{14} citric acid	3-C^{14}
2,4-C^{14} citric acid	2,4-C^{14}
2,3,4-C^{14} citric acid	2,3,4-C^{14}
2,3-C^{14} succinc acid	6,7-C^{14}
1,4-C^{14} succinic aicd	1,5-C^{14}

Two manuscripts are in preparation, the first concerning th prototype micro method for tropine labeling of atropine from arabinose and the second concerning the synthesis of N-Me C^{14} atropine.

Once N-Me-C^{14}-atropine was available, metabolic studies with the labeled drug, in mice, was initiated. At a dose of 100 mg/kilo, approximately 10% of the injected drug was metabolized to $C^{14}O_2$ which was not found in previous metabolic studies. This indicates that one possible metabolite is N-demethyl atropine or one of its derivatives. Chromatographic analysis, of the urine, revealed six major peaks. Of the injected dose, only 50 percent was recovered in expired air, urine, and feces. Since the remaining 50 percent of injected C^{14} is mostly in the animals, methods are being sought to determine the C^{14} remaining in the intact animals. Chromatographic analysis of the urine of mice receiving N-Me^{14} tropine in a dose of 180 mg/kilo revealed one major peak at Rf-.55 and two minor peaks.

In an effort to identify the tropine modified metabolites of atropine, metabolic studies will be conducted with each labeled atropine as they become available.

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13. ABSTRACT

During the first year, the investigation was directed to scaling down from 5 gm. to 300-400 mg level the synthesis of tropinone from succinic acid. Detailed studies for the formation of tropinone from precursor, conversion of tropinone to tropine and formation of atropine via the esterification of tropine with tropic acid was conducted; this work is published in the Journal of Pharmaceutical Science 56:215,1967. The primary objective of the second year was the labeling of atropine. Because of problems which arose in the reduction of tropinone; the original reduction method was discarded and a new procedure developed. After several abortive attempts, a small yield of N-Me-C^{14} tropine was obtained. A batch of N-Me-C^{14}-atropine has been prepared from the N-Me-C^{14}-tropine; this material has been used for some metabolic studies in mice, and part has been distributed to S.C.Kalser for metabolic studies in humans. Two attempts at making ring-labeled atropine are in progress. The 3-C^{14}, and the 2,3,4-C^{14}-atropine have been prepared, but the yields and specific activities were lower than anticipated. The remaining C^{14}-ring labeled atropines will be made in the next few months.

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